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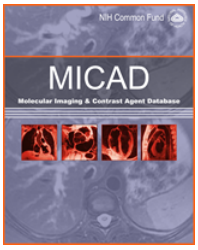

^{99m}Tc-Hexamethylpropyleneamine oxime-blue-biotin-liposomes

^{99m}Tc-HMPAO-BBL

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Chemical name:	^{99m}Tc-Hexamethylpropyleneamine oxime-blue-biotin-liposomes	
Abbreviated name:	^{99m}Tc-HMPAO-BBL	
Synonym:		
Agent category:	Small molecule (nanoparticle)	
Target:	Other	
Target category:	Other	
Method of detection:	Single-photon emission computed tomography (SPECT)	
Source of signal/contrast:	^{99m}Tc	
Activation:	No	
Studies:	• Rodents • Non-primate non-rodent mammals	No structure is currently available in PubChem.

Background

[PubMed]

The lymphatic system consists of a complex network of lymph vessels, organs, and lymph nodes, and it plays important roles in fluid homeostasis, toxin removal, and immune function (1). Lymph nodes are responsible for filtering lymph fluid and removing foreign materials such as bacteria and cancer cells (2). When lymph nodes become infected or are the sites of tumor metastases, they increase in size. Imaging of a sentinel node, the first lymph node into which the primary tumor drains, can facilitate diagnosis of nodal metastases in cancers (1). Thus, the development of imaging agents to target lymph nodes provokes a great deal of research interest. Colloids that are 2 to 200 nm in length are found to enter into lymphatics through clefts in the lymphatic walls or by endocytosis through the walls (2). After interstitial injection, colloids are transported to the nearest draining lymph node (the sentinel node). The colloids are retained through nonspecific mechanical trapping in the reticular meshwork of the node (2). The trapped colloids are released with time for subsequent migration to other downstream nodes. Lyposomes, a special type of colloid, are used in lymph node–targeted drug delivery

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and imaging (3). Encapsulation of imaging agents in liposomes increases lymph node targeting and yields enhanced detection sensitivity, for which only a small fraction of liposome-encapsulated labeling is cleared through the lymphatic vessels (3).

The ^{99m}Tc-hexamethylpropyleneamine oxime-blue-biotin-liposome (^{99m}Tc-HMPAO-BBL) is used for imaging lymphatic nodes with single-photon emission computed tomography (SPECT) (4). ^{99m}Tc-HMPAO-BBL consists of biotinylated lipids, liposome-encapsulated ^{99m}Tc-HMPAO complexes, and liposome-encapsulated blue dyes. ^{99m}Tc is a common radioactive label used in SPECT imaging (5), and it has a 0.90 gamma branch factor, a 6-h half-life at 140 keV, and a low isotope cost ($0.21/mCi) (5). The blue dye, a monosodium salt of 2,5-disulfonated triphenylmethane, has been approved by the United States Food and Drug Administration for clinical lymphangiography (4). HMPAO as a lipophilic chelator carries ^{99m}Tc across the lipid bilayer of preformed liposomes that contain reduced glutathione (GSH) (6). In the presence of GSH, ^{99m}Tc-HMPAO is chemically reduced to more hydrophilic molecules to be trapped within the liposomal aqueous space (6). This method provides stable ^{99m}Tc labeling that is retained within liposomes for prolonged periods without being metabolized. ^{99m}Tc-HMPAO-BBL targets lymphatic nodes through a retention mechanism (4) that requires co-injection of avidin, a 68-kDa glycoprotein that has an extraordinarily high affinity for biotin (~10^{15} M^{-1}) and can provide four biotin-binding sites. During migration through the lymphatic vessels, biotin-liposomes meet with the avidin, which is also moving into the lymphatic vessels. The association of the biotin-liposomes with avidin results in liposome aggregates. These liposomes aggregate and then become trapped in the first lymph node they encounter, thus prolonging their retention. This method can increase the accumulation of liposomes in lymph nodes from 1–2% to 12% (4). ^{99m}Tc-Labeled liposomes in combination with blue dye allow for additive identification of the sentinel lymph node.

Synthesis

[PubMed]

The synthesis of ^{99m}Tc-HMPAO-BBL liposomes was conducted in several steps (4). First, a dry film of liposomes was obtained by mixing lipids in chloroform followed by rotary evaporation and vacuum desiccation. The lipids included distearoyl phosphatidylcholine, cholesterol, *N*-biotinoyldistearoyl phosphoethanolamine, and α-tocopherol at a molar ratio (total lipid) of 58:39:1:2. Second, the dry film was treated first with a sucrose solution, then with a solution containing a reduced GSH and blue violet dye to produce soluble blue dye–encapsulated liposomes (BBL). This treatment was repeated several times to ensure sufficient encapsulation of sucrose, GSH, and blue dye into the liposomes. BBL had an average diameter of 136 nm and a phospholipid concentration of 29 mM. The intraliposomal concentration of blue dye was found to be 0.15 mg/ml in BBL, corresponding to an encapsulation efficiency of 1.5%. Finally, HMPAO was reacted with a solution containing 370 MBq (10 mCi) ^{99m}Tc-sodium pertechnetate. The produced ^{99m}Tc-labeled HMPAO was incubated with a concentrated suspension of BBL to yield ^{99m}Tc-HMPAO-BBL. The labeling efficiency was found to be 92.1 ± 1.9% by measuring the ^{99m}Tc activities with a dose calibrator.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

No publication is currently available.

Animal Studies

Rodents

[PubMed]

Phillips et al. conducted a biodistribution study of ^{99m}Tc-HMPAO-BBL in healthy rats (3). Experimental rats (n = 5; 200–300 g) received intraperitoneal injections of ^{99m}Tc-HMPAO-BBL (32.7 mg of phospholipid/kg and 43 MBq of ^{99m}Tc), followed by injections of 5 mg avidin (1 ml) in 30 min; the investigators reversed the order in the next two experiments. Control rats (n = 5, 200–300 g) only received ^{99m}Tc-HMPAO-BBL. After administration of ^{99m}Tc-HMPAO-BBL, whole-body dynamic scintigraphic images were collected per min for 60 min then at 24 h. For the experimental rats, increased accumulation of ^{99m}Tc activity was found in the abdominal and mediastinal nodes. Most of the activity was confined to the abdominal region, with virtually no uptake in the spleen. For the control rats, high activity appeared in the spleen, the organ that normally exhibits the highest liposome uptake. After 24 h, 81.9 ± 1.5% of the injected dose was still retained in the experimental animals compared with 72.7 ± 0.20% in the control animals. The distribution of ^{99m}Tc-HMPAO-BBL in tissues for the experimental and control rats was 0.17 ± 0.03% and 14.0 ± 1.7% in the blood and 0.78 ± 0.8% and 23.3 ± 3.9% in the spleen, respectively. The experimental group had additional distribution found in the abdominal nodes (4.7%) and mediastinal nodes (2.3%). After the imaging study, rats were euthanized for necropsy and tissue activity counting. The blue staining appeared clearly in the mediastinal and abdominal nodes in experimental rats; no blue-stained nodes were detectable in control rats. The total activity level in the blood and major organs (liver, spleen, kidneys, lungs, and blood) was 51.7% in the control rats *versus* 9.6% in the experimental rats, which suggested that ^{99m}Tc-HMPAO-BBL was easily cleared from the peritoneum and associated lymph nodes in the absence of avidin.

Zavaleta et al. examined regional uptake of ^{99m}Tc-HMPAO-BBL in rats with ovarian cancer xenografts (7). Athymic rats were injected with NIH:OVCAR-3 cells in the lower right peritoneal quadrant. The presence of ovarian cancer cells was validated 1 week later with ^{18}F-FDG positron emission tomography. Two weeks after inoculation, experimental rats (n = 4) received intraperitoneal injections of 0.3 ml avidin (5 mg) and then 2 ml ^{99m}Tc-HMPAO-BBL (38.4 mg of phospholipid/kg and 40.7 MBq of ^{99m}Tc) 2 h later. Control rats (n = 3) received only ^{99m}Tc-HMPAO-BBL. After injection of ^{99m}Tc-HMPAO-BBL, SPECT and computed tomography images were acquired at 4 and 22 h. At 4 h, retention of ^{99m}Tc-HMPAO-BBL appeared in the peritoneal cavity, the mediastinal nodes, and the two lymphatic channels that connect the peritoneal cavity and the mediastinal nodes. At 22 h, the experimental rats demonstrated continuous uptake of ^{99m}Tc-HMPAO-BBL in the peritoneal activity and the mediastinal nodes. The control rats demonstrated a different distribution pattern in that ^{99m}Tc-HMPAO-BBL was rapidly cleared from the peritoneal cavity and accumulated in the spleen at 24 h after injection. The biodistribution of ^{99m}Tc-HMPAO-BBL demonstrated that high amounts of uptake occurred in the diaphragm, mediastinal nodes, abdominal nodes, and omentum in the experimental rats. In comparison, liver and spleen uptake was minimal. The result was further confirmed by necropsy with the blue dye staining.

Other Non-Primate Mammals

[PubMed]

Phillips et al. tracked the migration of ^{99m}Tc-HMPAO-BBL toward lymphatic nodes in the presence of avidin in healthy rabbits (n = 6; 2.5–3.0 kg) (4, 8). Rabbits received subcutaneous injections of 0.3 ml ^{99m}Tc-HMPAO-BBL (1.5 mg phospholipid/kg and 12 ± 0.9 MBq of ^{99m}Tc) on the dorsum of each hind foot. Then 0.3 ml of 5 mg avidin was injected subcutaneously only on the right hind foot (experimental foot) ~2 cm proximal to the injection site of ^{99m}Tc-HMPAO-BBL. The left hind foot in each rabbit served as a control. Dynamic scintigraphic images were acquired at 1, 30, and 60 min and again at 24 h. The distribution of ^{99m}Tc-HMPAO-BBL was similar in the left and right popliteal lymph nodes at 30 min, but it was different at 60 min and 24 h. The deposition of ^{99m}Tc-HMPAO-BBL in the experimental popliteal node and the control popliteal node was 6.5% *versus* 1.7% at 60 min and 8.5% *versus* 1.0% at 24 h. The retention efficiency in the experimental leg and the control leg was 20.1% *versus* 8.5% at 60 min and 17.0% *versus* 2.5% at 24 h. This was further confirmed with necropsy and radioactivity counting at 24 h after completion of imaging study. In addition, the experimental popliteal node was stained a deep blue color compared to a minimal blue coloration on the control popliteal

node. Tissue biodistribution (as percent injected dose per gram (%ID/g)) demonstrated a highly significant increase in liposome deposition in the experimental popliteal node compared with the control popliteal node: 12.2 ± 1.5% ID/g *versus* 1.2 ± 0.1% ID/g, or 71.4 ± 12.4% ID/g *versus* 7.4 ± 1.8% ID/g, respectively.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

NIH Support

EB 000817

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